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Short Communication

Prediction of Progression from Refractory Cytopenia with Unilineage Dysplasia by Analysis of Bone Marrow Blast Cell Composition

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A retrospective analysis of 71 patients newly diagnosed with refractory cytopenia with unilineage dysplasia (RCUD) revealed that 12 developed refractory anemia with an excess of blasts or acute myeloblastic leukemia. Before the diagnosis of RCUD was made, phenotypes of cells in the bone marrow (BM) blast region were analyzed using flow cytometry. Patients with RCUD were divided into two groups ; those with no progression (Group A) and those with disease progression later on (Group B). The cell composition in the BM blast region differed significantly between the groups : Group A showed higher percentages of B lymphoid cells but lower percentages of myeloid cells. A cut-off value of 20 for the CD33/CD10 ratio in the BM blast region clearly separated Group A from Group B. These results suggest that cell composition in the BM blast region evaluated by flow cytometry may indicate the progression of RCUD. 〔*J Clin Exp Hematopathol 52(1) : 63-66, 2012*〕

Keywords: myelodysplastic syndrome, bone marrow, flow cytometry, blast

INTRODUCTION

Refractory anemia (RA) is a type of myelodysplastic syndrome (MDS), which was first defined in the French, American, and British classification.[1] In the revised (2008) World Health Organization (WHO) classification, RA was reclassified into refractory cytopenia with unilineage dysplasia (RCUD), which includes several cytopenias.[2] Gene expression arrays showed that the progression of MDS from RA to advanced phases is associated with the aberrant expression of genes associated with proliferation and differentiation.[3] In a clinical setting, simplified methods to predict the progression from RCUD are needed. We analyzed the cell composition in a blast cell region of the bone marrow (BM) in RCUD patients by employing flow cytometry (FCM).

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PATIENTS AND METHODS

During the period from June 1996 to February 2008, 71 patients who were newly diagnosed with RA according to the WHO classification (2002) were reclassified according to the revised WHO classification (2008).[2] Three-color FCM combined with two-color FCM was performed to evaluate the phenotypes of cells in the BM blast region on initial presentation (Table 1).[4] Blasts are characterized by intermediate CD45 expression and low side scatter properties and most of the cells in the region are lymphoblasts and myeloblasts.[4] Stained cells were analyzed using a flow cytometer (FACSCalibur ; BD Biosciences, San Jose, CA, USA). Progression was defined as the appearance of blasts in the peripheral blood (PB) after the diagnosis of RCUD. Once blasts appeared in the PB, BM aspiration was performed to determine whether the disease progressed to advanced stages. Follow-up was conducted until determination as progression or a recent examination of PB in which blasts were not observed. The follow-up period ended on February 1, 2009. Therapy was started on the basis of doctors' decisions regarding their patients' conditions. *P*-values below 0.05 were considered significant using Student's *t*-test and the χ^2 test.

RESULTS AND DISCUSSION

Patients who did not show disease progression were categorized as Group A, while patients who showed disease progression were categorized as Group B. In Group A, 44, 11, and 4 patients were diagnosed as having RA, refractory neutropenia, and refractory thrombocytopenia, respectively. In Group B, 8, 2, and 2 patients were diagnosed as having RA, refractory neutropenia, and refractory thrombocytopenia, respectively. Of the 59 patients with RCUD in Group A, 6 patients had been incorporated in the previously reported study.[4] In Group B, disease progression was confirmed by BM aspiration as follows : 7 patients, RA with excess blasts-1 ; 4, RA with excess blasts-2 ; and 1, overt leukemia. The median follow-up periods in Groups A and B were 36 and 11 months, respectively. As shown in Table 2, there were no differences between the two groups in terms of age, sex, PB cell count, BM blast percentage, chromosomal risk factors, and International Prognostic Scoring System (IPSS) scores. By the end of the follow-up, about half of the patients in Group A had not received any medications including cyclosporine, steroid, anabolic steroid, antithymocyte globulin, or a combination thereof. In Group B, only 3 of 12 patients underwent watchful waiting, the other patients receiving the medications described above. In the flow cytometric analysis, Group A showed higher percentages of B lymphoid cells but lower percentages of myeloid cells in the BM blast region than Group B (Table 2, Fig. 1). The percentages of $CD34^+$ and $CD117^+$ cells in the BM blast region were significantly higher in Group B than in Group A ($p < 0.05$). There were no differences between the two groups in the percentages of $CD15^+$, $CD14^+$, $CD11b^+$, $CD11c^+$, $CD5^+$, $CD2^+$, $CD7^+$, $CD25^+$, $CD36^+$, $CD41^+$, and HLA-DR^+ cells in the region (data not shown). To enhance the balance of myeloid cells and B lymphoid cells in the region, ratios for the myeloid cell percentages to the B lymphoid cell percentages were calculated ; significant differences in CD33/CD19, CD33/CD10, CD13/CD19, and CD13/CD10 ratios were noted between the

Table 1. Antibody combinations

PE	FITC	PerCP
Control	Control	CD45
CD34	CD7	CD45
CD13	CD10	CD45
CD33	CD19	CD45
CD117	CD5	CD45
CD56	CD2	CD45
	CD41	CD45
	CD11b	CD45
	CD11c	CD45
	CD14	CD45
	CD15	CD45
	CD20	CD45
	CD25	CD45
	CD36	CD45
	CD235a	CD45
	HLA-DR	CD45
	MPO	CD45
	TdT	CD45

PE, phycoerythrin ; FITC, fluorescein isothiocyanate ; PerCP, peridinin chlorophyll protein ; MPO, myeloperoxidase ; TdT, terminal deoxynucleotidyl transferase

Table 2. Characteristics of the patients

Clinical findings	Group A	Group B	*p*
Patients (no.)	59	12	
Age (years)[#]	65 (35-87)	64 (45-77)	0.685
Sex (no. ; male/female)	33/26	7/5	0.878
White blood cells ($\times 10^9$/L)[#]	3.0	3.2	0.793
Hemoglobin (g/dL)[#]	9.3	7.8	0.186
Platelets ($\times 10^9$/L)[#]	141	134	0.833
BM blasts (%)[#]	2.9	2.8	0.630
IPSS chromosome (no.)			0.608
Good	51	11	
Intermediate	6	0	
Poor	2	1	
IPSS score (no.)			0.387
Low	31	3	
Intermediate-1	28	9	
Intermediate-2	0	0	
High	0	0	
Phenotypes of the cells in the BMBCC (%)[#]			
$CD19^+$	27.7	7.3	< 0. 001
$CD10^+$	14.4	4.2	< 0. 01
$CD13^+$	51.2	68.4	< 0. 05
$CD33^+$	64.4	78.2	< 0. 01
$CD34^+$	40.6	60.9	< 0. 05
$CD117^+$	24.0	59.7	< 0. 001
Ratios of the cells in the BMBCC[#]			
CD33/CD19	3.2 (0.8-4.8)	23.1 (20.5-37.1)	< 0. 01
CD33/CD10	3.2 (0.9-5.8)	31.8 (27.8-42.6)	< 0. 001
CD13/CD19	3.0 (0.5-5.0)	21.9 (11.6-32.7)	< 0. 001
CD13/CD10	2.5 (0.6-6.3)	39.9 (19.2-50.8)	< 0. 001
Treatment (no.)			< 0. 05
No medications	35	3	
Medications	24	9	

Group A, no progression after the diagnosis of refracory anemia ; Group B, progression after that ; no., number ; #, mean ; BM, bone marrow ; IPSS, International Prognostic Scoring System ; BMBCC, bone marrow blast cell composition ; Medications include cyclosporine, steroid, anabolic steroid, antithymocyte globulin, or a combination thereof.

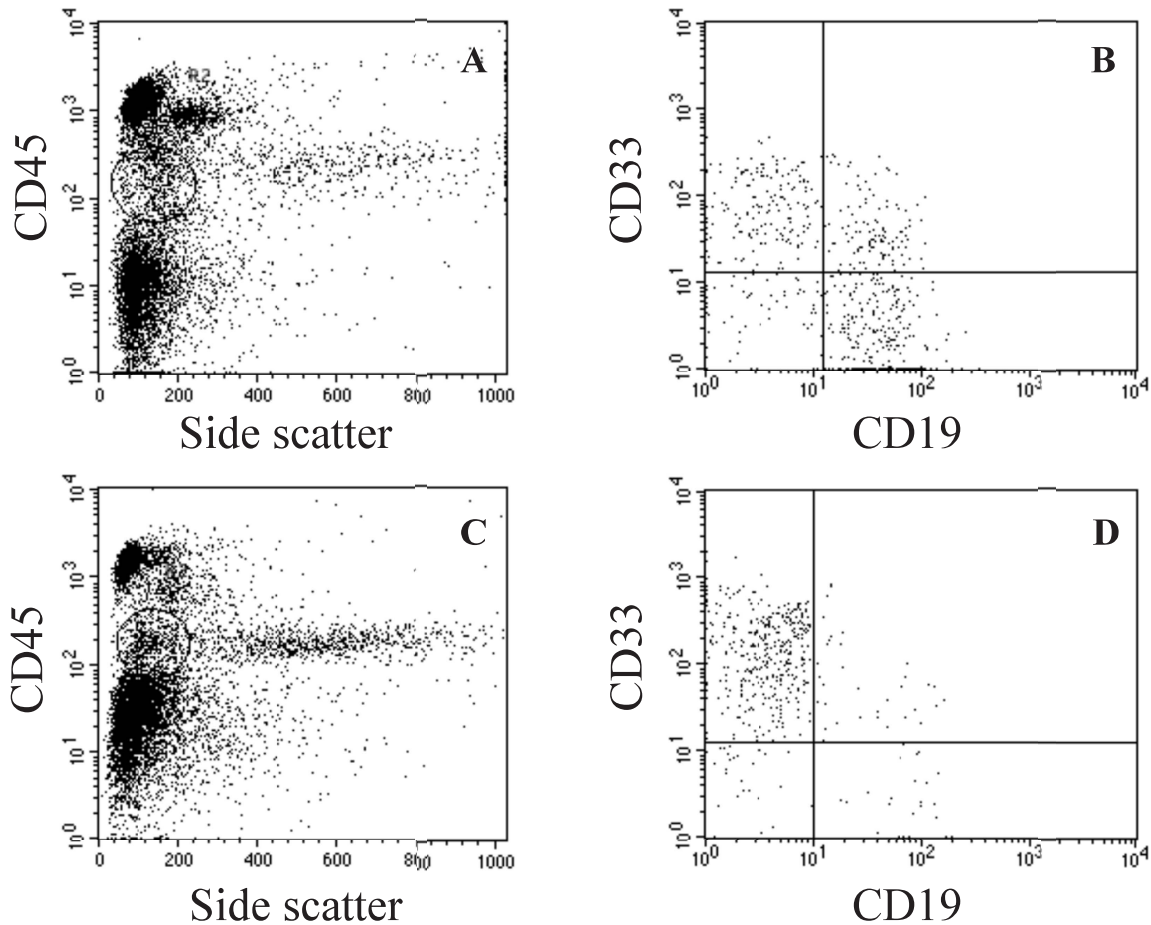

Fig. 1. Flow cytometric analysis of bone marrow cells. (*1A*) & (*1B*), a case in Group A ; (*1C*) & (*1D*), a case in Group B. The circle indicates the bone marrow (BM) blast gate region. The cell composition in the region is different between the two patients. Co-expression of CD34 and CD117 in the BM blasts was not measured.

two groups (Table 2, Fig. 2). Differences in the mean values of CD33/CD10 ratios between the two groups were large and variations of the ratios in Group B were small. A cut-off value of 20 for the CD33/CD10 ratio clearly separated Group A from Group B. At the end of October 2011, patients in Group A and Group B were evaluated by medical charts. In Group A, 22 patients were alive, 4 patients died due to ovarian cancer, cardiac sudden arrest, hepatoma or cholecystitis, and 33 patients were lost to follow-up. Only one patient underwent bone marrow transplantation and she was alive. In Group B, 3 patients were alive, 6 patients died due to hypoglycemia, pneumonia or sepsis, and 3 patients were lost to follow-up. All of the surviving patients underwent bone marrow transplantation.

Abnormalities in antigen expression in $CD34^+$ cells have been reported, such as the overexpression of CD33, CD117, and HLA-DR, asynchronous expression of CD11b, CD15, and CD16, and aberrant expression of CD7 and CD56.[5] Tavil *et al*. reported that an increase of the $CD34^+$ $CD117^+$ cells in BM blasts is associated with MDS progression.[6] Abnormal antigen expression has been detected in granulocytes, monocytes, and erythroblasts.[5,7,8] Recently, Xu *et al*. reported a flow cytometric scoring system for the diagnosis of MDS.[9] To identify abnormalities in antigen expression in MDS patients by FCM, it is necessary to have a clear understanding of antigen expression profiles along with normal hematopoietic stem cell differentiation, complicating the use of FCM for the diagnosis of MDS as a routine practice. It is not clear whether abnormal antigen expression profiles in MDS can be used to predict the progression of the disease. Previously, Stemberg *et al*. showed that B precursor cells in the BM of patients with MDS decrease in number.[10] This report suggests that B lymphoid cells in the BM blast cell region evaluated by FCM reflect disease progression from RCUD. Taking these findings together, CD33 and CD10 ratios in the BM blast region may be useful markers for predicting the

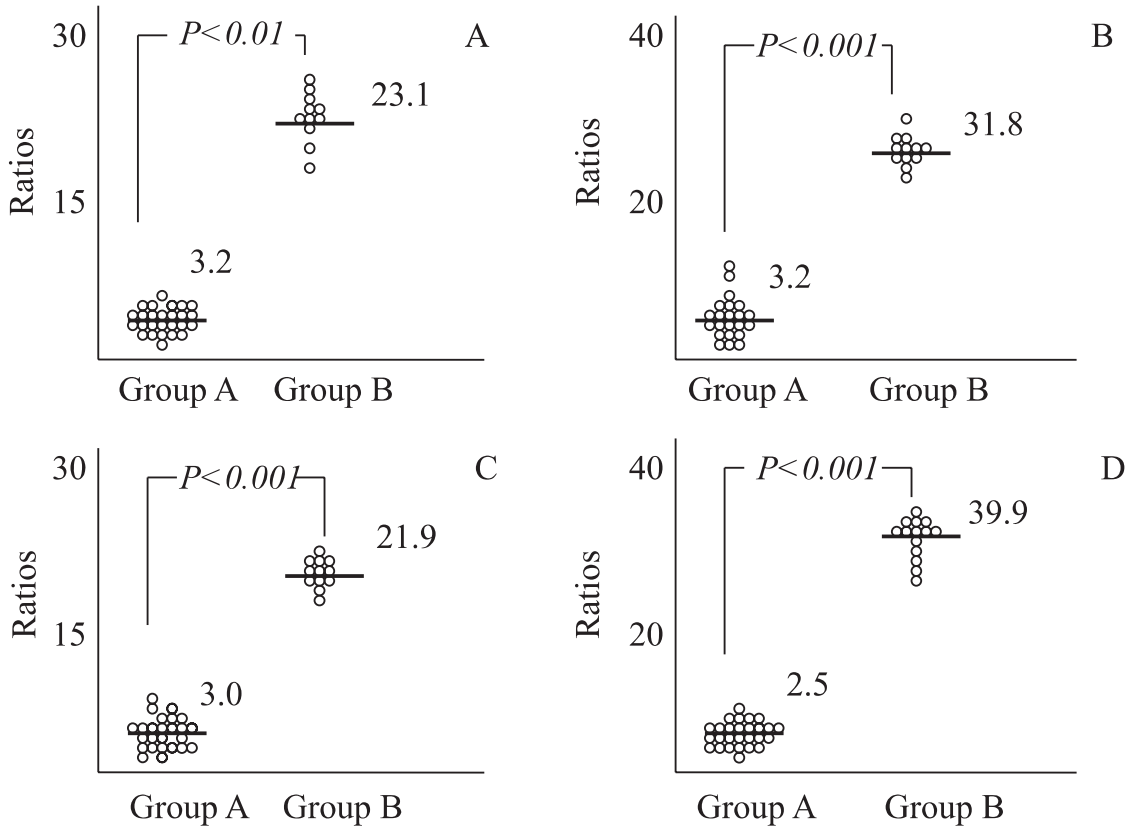

Fig. 2. Ratios of antigen expression in the blast region of bone marrow. (***2A***), CD33/CD19 ; (***2B***), CD33/CD10 ; (***2C***), CD13/CD10 ; (***2D***), CD13/CD19. The values are means.

progression of RCUD. To confirm our results, prospective studies are needed.

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